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Allelic Association But Only Weak Evidence for Linkage to the Apolipoprotein E Locus in Late-Onset Swedish Alzheimer Families

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An association between the ε4 allele of the apolipoprotein E gene (APOE) and late-onset Alzheimer's disease (AD) was recently demonstrated. In order to confirm the association and to gauge the ability of standard genetic linkage methods to identify susceptibility genes, we investigated 15 Swedish late-onset AD families. We found an association of familial AD to the APOE ε4 allele (P = 0.01) but no indication of linkage to the APOE region using 2-point linkage analysis, and only weak evidence using the affected pedigree-member (APM) method. Our results confirm an APOE ε4 association with late-onset familial AD and indicate that susceptibility genes can easily be missed when using standard lod score and APM genetic linkage analysis.

KEY WORDS: Alzheimer's disease, apolipoprotein E, allelic association, linkage analysis, polymorphism, chromosome 19

INTRODUCTION

Apolipoprotein E (apoE) is involved in the transport of lipids and plays a key role in nerve cell regeneration following injury [Boyles et al., 1985]. ApoE has been found as one of the components of senile plaques and neurofibrillary tangles in Alzheimer's disease [AD; Wisniewski and Frangione, 1992]. It has three isoforms, E2, E3, and E4 that differ by one or 2 amino acids, encoded by 3 alleles, ε2, ε3, and ε4 at the apolipoprotein E locus (APOE) on chromosome 19. Recently, an association was reported between APOE ε4 and late-onset (>age 60 years) familial and sporadic AD. The ε4 allele frequency was 50% in late-onset familial cases as compared to 16% in controls [Strittmatter et al., 1993; Saunders et al., 1993]. The risk for AD is increased by a factor of 3 with one copy of the ε4 allele and 8 times in ε4 homozygotes [Corder et al., 1993]. Furthermore, there was weak evidence (lod score = 1.85) for genetic linkage of APOE and late-onset AD in at least one family reported in the literature, suggesting that standard linkage methods might detect susceptibility genes in at least some instances [Borgaonkar et al., 1993]. To confirm the association of APOE ε4 with late-onset familial AD and to gauge the ability of standard genetic linkage methods to identify susceptibility genes, we evaluated the association and genetic linkage of AD to the APOE locus using 2-point linkage and affected pedigree-member (APM) methods in 15 Swedish late-onset AD families.

HUMAN SUBJECTS, MATERIALS, AND METHODS

Families

Fifteen late-onset AD families (mean age of onset >60 years) with at least 3 diseased individuals in each family, within a total of 44 patients and 151 healthy or at-risk members, were investigated (Table I, Fig. 1). All

TABLE I. Clinical Information on AD Families

Family number	Mean age of onset	No. of generations	No. of affected
F100	69	3	4
F102	73	4	3
F104	77	4	4
F106	72	3	6
F107	65	2	6
F118	71	3	7
F133	69	3	8
F151	68	4	5
F163	71	3	5
F164	63	3	4
F175	64	4	4
F176	68	5	7
F197	67	3	4
F204	71	3	5
F205	70	4	6

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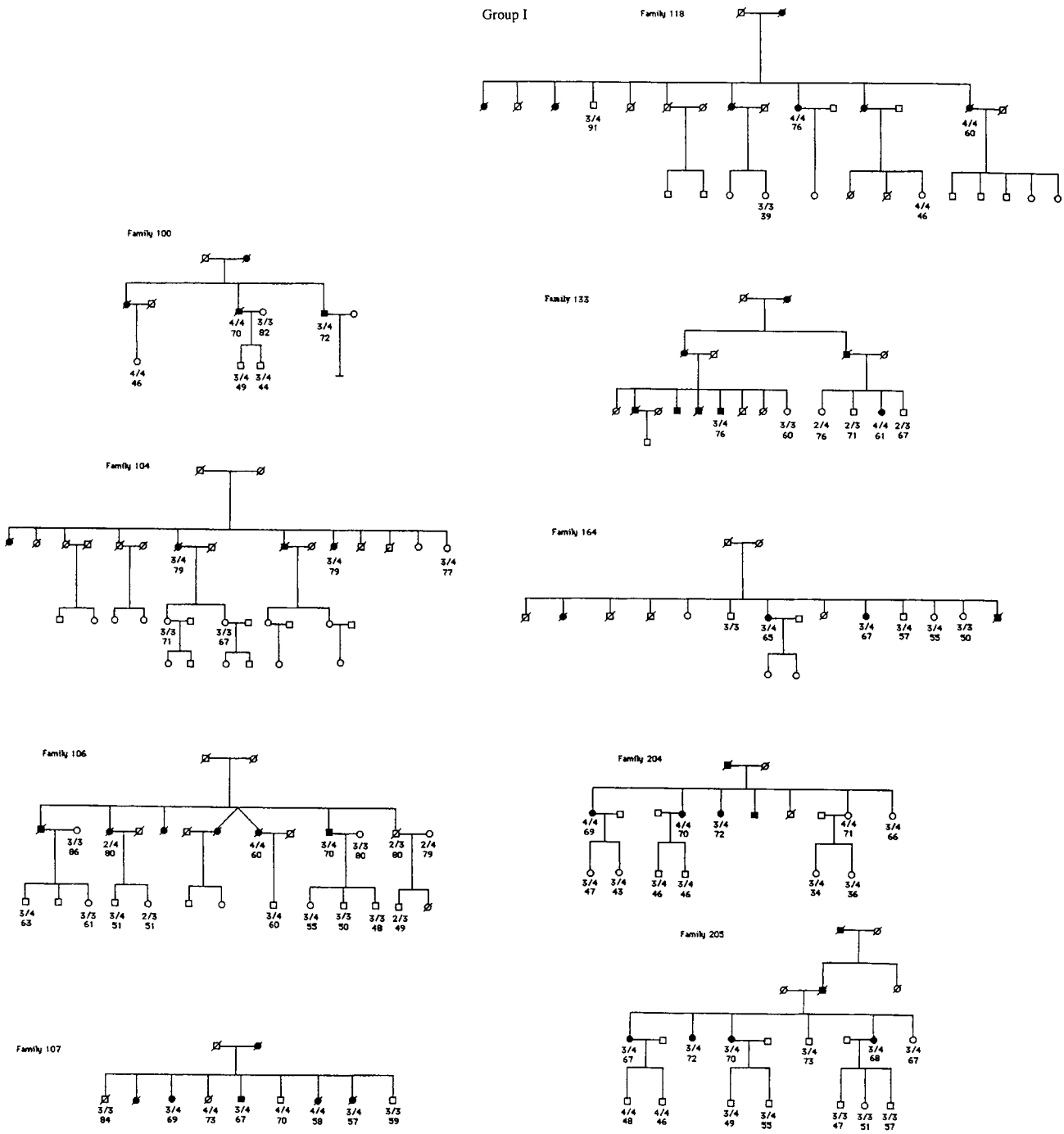

Fig. 1. Pedigrees of 15 AD families divided into 3 groups are shown, according to the distribution of the ϵ4 allele. Symbols: squares = male; circles = female. Filled symbols are affected individuals, half-filled symbols are individuals with unknown phenotype, and slashed symbols represent deceased individuals. APOE genotypes are given under the symbols. Age of onset is given for diseased individuals and present age is shown for unaffected individuals.

affected individuals were diagnosed as possible AD according to NINCDS-ADRDA criteria [McKhann et al., 1984]. Other diseases with progressive memory deficits were excluded on clinical grounds, like depressive disorder, Parkinson's disease, multi-infarct dementia, and drug intoxication. Five postmortem autopsy confirmations of AD were obtained in 4 of these families. Information concerning hospitalization, presence of dementia, date of disease onset, and its course was collected from medical records and by interviewing relatives. The age at onset of dementia was defined when either memory loss or a change in behavior was first observed by relatives and then diagnosed as AD by careful medical examination.

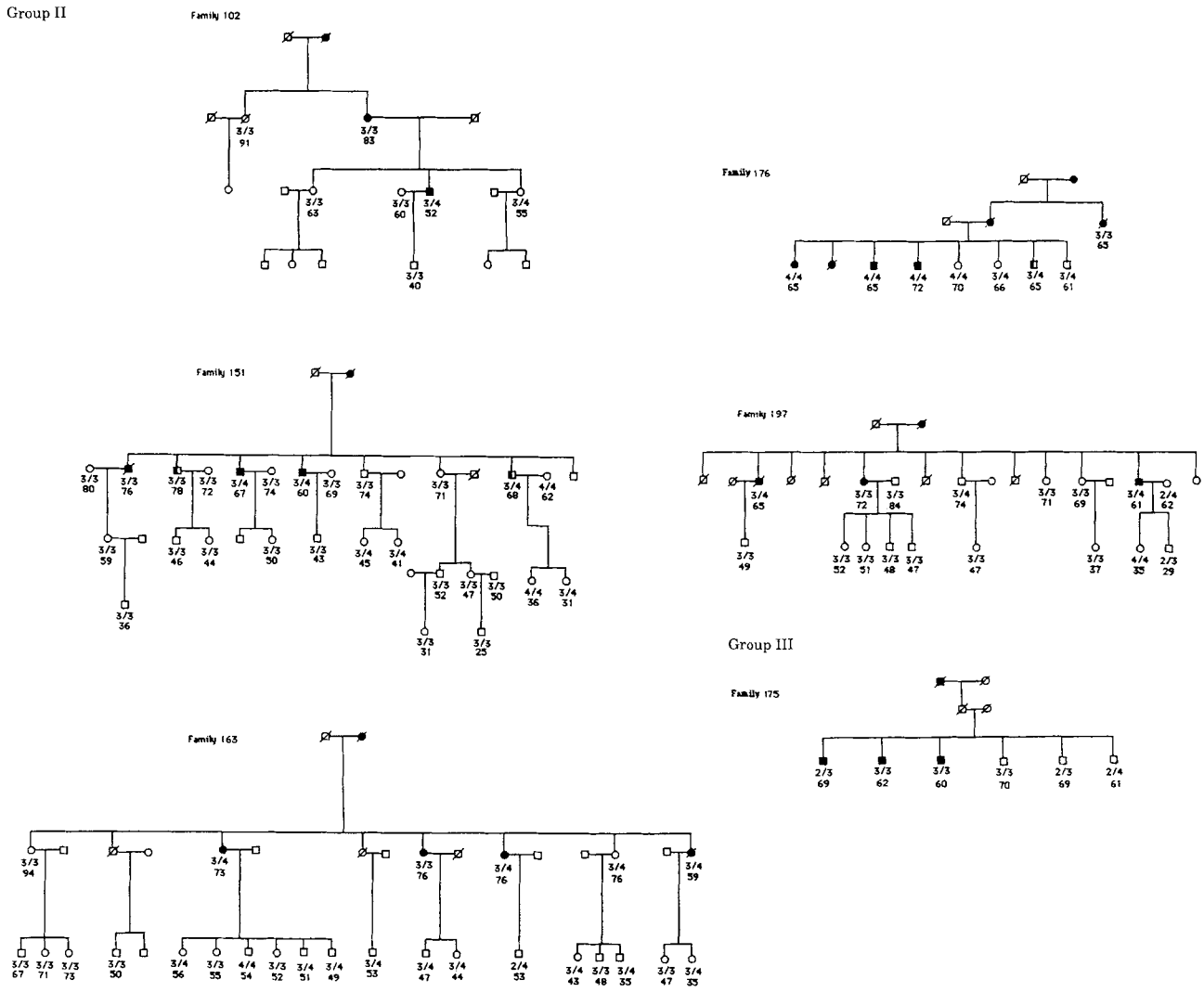

Fig. 1. (*continued*).

Polymerase Chain Reaction and Identification of APOE Genotypes

DNA was prepared from peripheral blood [Higuchi, 1989] and the APOE genotype was determined by the method of Wenham et al. [1991] with some minor modifications. The person running the assay was blinded to AD status.

Association Study

To investigate association between the $\epsilon 4$ allele and late-onset AD, the Bayesian extension to the transmission disequilibrium test (TDT) was used, a family-based association method. TDT looks at transmission of candidate alleles from heterozygous parents to affected offspring. Missing parental alleles are inferred from affected and unaffected offspring [Corder et al., 1994].

Linkage Analysis

Two-point standard lod score and APM genetic linkage analysis were performed in the 15 AD families. The influence of $\epsilon 4$ on linkage results was explored by dividing the families into 3 groups based on the number of $\epsilon 4$ alleles in affected family members. In group I consisting of 9 families, all 24 affected subjects had one or 2 $\epsilon 4$ alleles. In group II consisting of 5 families, 11 of 15 affected members were carriers of one or 2 $\epsilon 4$ alleles. Finally, in group III, consisting of only one family, none of the affected members had the $\epsilon 4$ allele.

Mlink from the linkage package (version 5.1) was used to perform 2-point linkage analysis at each of the following recombination fractions 0.00, 0.05, 0.10, 0.15, 0.20, and 0.30 (θ males = θ females) [Ott, 1991]. A single-locus model with an autosomal dominant inheritance was assumed, which was compatible with the inheritance as it appeared in the pedigrees. A cumulative

TABLE II. Age-Dependent Penetrances

Age (years)	Disease gene DD, Dd	Phenocopy dd
30–50	0.001	0.90
51–60	0.04	0.001
61–65	0.20	0.01
66–70	0.40	0.01
71–75	0.60	0.02
76–80	0.80	0.02
>80	0.95	0.05

age-dependent penetrance was assigned as shown in Table II [Farrer et al., 1990; Goldin and Gershon, 1993]. Individuals were put into different liability classes depending on the age at onset (affected) or age at last examination (unaffected). The disease gene frequency was estimated to be 0.001 and the marker allele frequencies were $\epsilon 2 = 0.09$, $\epsilon 3 = 0.73$, and $\epsilon 4 = 0.18$ [Lannfelt et al., 1994].

The APM method [Weeks and Lange, 1988] gives test statistics for 3 different weighting functions: $f(p) = 1$, $f(p) = 1/\sqrt{p}$, $f(p) = 1/p$, where p is the allele frequency. We report the results from the intermediate function, since the first is too conservative and the third usually leads to nonnormality of the statistics. The P values were empirically estimated.

RESULTS

There was evidence for an association of APOE $\epsilon 4$ with AD in the 15 Swedish AD families. TDT gave odds ratios of 2.2 ($P = 0.01$) and 2.1 ($P = 0.02$) for transmission of $\epsilon 4$ to affected offspring instead of $\epsilon 2$ or $\epsilon 3$ or just $\epsilon 3$, respectively (Table III).

No linkage of AD to the APOE locus was found calculating the 15 families together with 2-point linkage ($Z = -7.00$), and APM analysis gave no evidence for linkage with test statistic of 1.20 and an empirical P value of 0.12 (Table IV).

Consistent with the results of the summed 2-point lod scores for all 15 families, there was no evidence for genetic linkage of AD to the APOE locus in the 3 groups. In group I with 9 families, the summation of lod scores was negative ($Z = -1.81$; Table IV). In group II with 5 families, the sum of lod scores was significantly negative ($Z = -4.57$; Table IV). Finally, in group III, consisting of family 175, the lod score was -0.62 (Table IV).

The APM method showed evidence for linkage only in group I with a test statistic of 2.28 ($P = 0.02$), a result mainly coming from families 118 and 204, but failed to give significant results for the other 2 groups with test statistics of -0.57 ($P = 0.69$) and -0.56 ($P = 0.56$), respectively (Table IV).

DISCUSSION

In the present study we investigated the association and distribution of the $\epsilon 4$ allele of APOE in 15 late-onset AD families. Furthermore, we explored whether standard genetic linkage methods are likely to identify a susceptibility gene like APOE, which shows allelic association with AD.

Even in this limited number of 15 AD families, association between the $\epsilon 4$ allele and AD was found ($P = 0.01$) with TDT. Despite this allelic association, the 2-point linkage and APM methods did not give strong evidence for linkage. These 2 methods calculate linkage in different ways. Two-point analysis detects linkage by testing for cosegregation of the disease with a marker allele and the APM tests if affected individuals are more similar at a locus than expected by chance. APM may be useful for analyzing complex diseases since it is independent of the mode of inheritance and can avoid the potentially false-negative results from model misspecification in the 2-point linkage analysis. In addition, APM, which is programmed to use identity by state rather than identity by descent, may detect susceptibility loci but relatively small changes in the frequency of rare alleles may significantly change the result [Babron et al., 1993; Yu et al., 1994].

The results of the linkage approaches in the families grouped according to the distribution of the $\epsilon 4$ alleles show similar pattern as for all families together. In group I, where all diseased individuals carried at least one $\epsilon 4$ allele, the 2-point lod score was negative as several healthy individuals in these families also were $\epsilon 4$ carriers. In agreement with this, many cognitive normal very old $\epsilon 4/4$ allele carriers were recently described in a population-based study [Corder et al., 1995]. However, spurious negative lod scores might result from $\epsilon 4$ carriers without signs of cognitive impairment that will develop AD later. In this group of families it could be argued that a pathogenic effect of the apoE4 protein might be operating but with a reduced penetrance. In group II, most but not all AD cases had the $\epsilon 4$ allele. Affected members without the $\epsilon 4$ allele might be phenocopies with a different etiology, which could explain the negative lod score found in these families. In group III, consisting of only family 175, the lod score was not significant as all patients were $\epsilon 3$ carriers and the $\epsilon 3$ allele is very frequent (Fig. 1, Table

TABLE III. Transmission Disequilibrium Test of APOE in Affected and Unaffected Offspring

Comparison	No. of transmitted alleles		Odds ratio[a]		Probability		Confidence interval	
	Affected	Unaffected	Affected	Unaffected	Affected	Unaffected	Affected	Unaffected
$\epsilon 4$ vs $\epsilon 2$ or $\epsilon 3$	35.0:15.7	11.8:23.4	2.2	0.5	0.01	ns	1.23–4.01	0.25–1.02
$\epsilon 4$ vs $\epsilon 3$	30.9:14.7	11.8:19.4	2.1	0.6	0.02	ns	1.13–3.91	0.29–1.25
$\epsilon 4$ vs $\epsilon 2$	4.2:1.0	1.1:4.0	4.2	0.3	ns	ns	0.47–37.0	0.03–2.25
$\epsilon 3$ vs $\epsilon 2$	2.1:1.0	—	2.1	—	ns	ns	0.19–22.7	—

[a] The $\epsilon 4$ allele is transmitted more often than the $\epsilon 2$ or $\epsilon 3$ alleles to affected offspring.

TABLE IV. Two-Point Lod Scores at θ = 0 and APM Test Statistics for the APOE Polymorphisms vs. AD

	Families	Two-point lod scores	APM test statistics* f(p) = 1/√p
All families		−7.00	1.20 (p = 0.12)
Group I	F100	0.15	0.65 (ns)
All affected subjects	F104	−0.02	−0.16 (ns)
carry the ε4 allele	F106	−1.14	0.15 (ns)
	F107	−0.08	0.35 (ns)
	F118	−0.58	3.86 (p < 0.001)
	F133	0.44	1.07 (ns)
	F164	0.02	−0.16 (ns)
	F204	0.23	2.21 (p = 0.02)
	F205	−0.83	−0.23 (ns)
		Σ −1.81	Σ 2.28 (p = 0.02)
Group II	F102	0.18	—[a]
Some affected subjects	F151	−0.40	−0.89 (ns)
carry the ε4 allele	F163	−0.89	−0.81 (ns)
	F176	−3.02	1.22 (ns)
	F197	−0.44	−0.90 (ns)
		Σ −4.57	Σ −0.57 (p = 0.69)
Group III			
No affected subjects carry the ε4 allele	F175	−0.62	−0.56 (p = 0.56)

[a] Family 102 was not possible to analyze with APM since the affecteds were a parent and a child.
* The accuracy of the APM statistic depends on having a large number of families. Hence, we report the empirical *P* values.

IV). A conclusion is that APOE ε4 alleles do not correlate well with affection status in late-onset families, and thus has a poor predictive ability [Bennett et al., 1995].

When the APM test statistic was calculated for all 15 families, a nonsignificant test statistic of 1.20 was achieved with an empirically estimated *P* value of 0.12. The APM method showed evidence for linkage in the first group where the test statistic was 2.28 ($P = 0.02$) as all affected were ε4 carriers. However, linkage was only found in 2 kindreds (families 118 and 204; Table IV), where several affected were ε4 homozygotes (Fig. 1). APM failed to produce significant results for the other 2 groups of families.

In conclusion, we found allelic association of the ε4 allele to late-onset familial AD, consistent with previous reports. However, 2-point linkage failed to identify this susceptibility gene. APM gave evidence for linkage only in group I. Our study indicates that susceptibility genes can easily be missed, using 2-point and APM genetic linkage methods.

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